## **CLAIMS:**

- 1. A method for detecting L-ficolin dependent activation of the lectin pathway of complement comprising:
- (a) contacting L-ficolin lectin pathway activation complex with Lipoteichoic acid (LTA) in conditions that permit specific binding thereof, and
  - (b) detecting complement activation.
- 2. A method according to claim 1 wherein LTA is immobilised on a support.
- 3. A method according to claim 1 or claim 2 wherein L-ficolin complex is obtained from blood.
- 4. A method according to any preceding claim wherein complement activation is detected by a C3 and/or C4 cleavage assay.
- 5. A method according to claim 4 wherein complement activation is detected by detection of a C3 and/or a C4 cleavage product.
- 6. A method according to claim 4 or 5 wherein the C3 and/or C4 cleavage product is detected using a ligand specific for the cleavage product, labelled directly or indirectly with a detectable marker.
- 7. A method according to claim 6 wherein the ligand specific for the cleavage product is an antibody or a binding fragment of an antibody.
- 8. A method according to any preceding claim wherein complement activation is detected by detection of the C3 cleavage product C3b.
- 9. A method according to claim 8 wherein the ligand is an anti-C3b antibody or a binding fragment of an anti-C3b antibody.

10. A method according to any preceding claim wherein complement activation is detected by detection of the C4 cleavage product C4b.

24

- 11. A method according to claim 10 wherein the ligand is an anti-C4b antibody or a binding fragment of an anti-C4b antibody.
- 12. A method according to any preceding claim wherein complement activation is detected by detection of the C4 cleavage product C4c.
- 13. A method according to claim 12 wherein the ligand is an anti-C4c antibody or a binding fragment of an anti-C4c antibody.
- 14. A method according to any one of claims 6 to 13 wherein the detectable marker is a fluorescent, luminescent or radioactive marker.
- 15. A method according to any one of claims 6 to 14 wherein the detectable marker is selected from the group comprising alkaline phosphatase, horse radish peroxidase, biotin, europium, fluorescein isothiocyanate, a fluorescent protein or a radiolabel.
- 16. A method according to any one of claims 6 to 15 wherein the detectable marker is alkaline phosphatase and the alkaline phosphatase is detected using a colorimetric substrate, preferably p-nitrophenyl phosphate (pNPP).
- 17. A method according to any one of claims 6 to 15 wherein the detectable marker is fluorescein isothiocyanate (FITC) and is detected using fluorescence microscopy.
- 18. An assay for L-ficolin dependent activation of the lectin pathway of complement, comprising a method according to any one of claims 1 to 17.
- 19. A method for identifying an L-ficolin abnormality comprising a method or assay according to any preceding claim.

- 20. A method for identifying an L-ficolin abnormality comprising:
- (a) contacting LTA with a solution comprising blood, serum or an extract therefrom, in conditions that permit specific binding of L-ficolin lectin pathway activation complex to LTA, and,
- (b) detecting, and optionally quantifying, specific binding of the L-ficolin complex to LTA.
- 21. A method according to claim 20 wherein LTA is immobilised on a support.
- 22. A method for detecting and/or identifying gram positive bacteria comprising:
- (a) contacting a sample comprising bacteria or suspected of comprising bacteria with L-ficolin complex in conditions that permit specific binding of L-ficolin to LTA, and,
- (b) detecting specific binding of L-ficolin complex to LTA present on gram positive bacteria.
- 23. A method according to any one of claims 20 to 22 wherein specific binding of L-ficolin complex to LTA is detected using a ligand labelled directly or indirectly with a detectable marker.
- 24. A method according to claim 23 wherein the ligand is an antibody or a binding fragment of an antibody.
- 25. A method according to claim 24 wherein the antibody is an antibody specific for L-ficolin or a binding fragment of an antibody specific for L-ficolin.
- 26. A method according to claim 25 wherein the antibody is GN4 or GN5 or a fragment thereof that specifically binds L-ficolin.

- 27. A method according to any one of claims wherein the detectable marker is a fluorescent, luminescent or radioactive marker.
- 28. A method according to any one of claims 23 to 27 wherein the detectable marker is selected from the group comprising alkaline phosphatase, horse radish peroxidase, biotin, europium, fluorescein isothiocyanate, a fluorescent protein or a radiolabel.
- 29. A method according to claim 28 wherein the detectable marker is alkaline phosphatase and the alkaline phosphatase is detected using a colorimetric substrate, preferably p-nitrophenyl phosphate (pNPP).
- 30. A method according to claim 28 wherein the detectable marker is fluorescein isothiocyanate and is detected using fluorescence microscopy.
- 31. A method or assay according to any one of claims 1 to 30 performed in multiwell format, preferably 96 well format.
- 32. A method or assay according to any one of claims 1 to 31 performed in high throughput format.
- 33. A kit for performing a method or assay according to any one of the preceding claims.
- 34. A kit according to claim 33 for detecting L-ficolin dependent activation of the lectin pathway comprising:
  - (a) LTA immobilised on a support,
  - (b) a purified C4 or crude C4/C3 preparation, and
  - (c) a reagent or reagents for detection of a C3 and/or C4 cleavage product, and
  - (d) optionally standard serum or purified L-ficolin/MASP complex suitable for generation of a standard curve, and,
  - (e) optionally instructions for use of the kit.

- 35. A kit according to claim 34 wherein a reagent for detection of a C3 and/or C4 cleavage product comprises a ligand capable of being labelled directly or indirectly with a detectable marker.
- 36. A kit according to claim 35 wherein the ligand is an antibody or a binding fragment of an antibody.
- 37. A kit according to claim 35 or 36 wherein the ligand specifically binds the C3 cleavage product C3b.
- 38. A kit according to any one of claims 35 to 37 wherein the ligand specifically binds the C4 cleavage product C4b.
- 39. A kit according to any one of claims 35 to 38 wherein the ligand specifically binds the C4 cleavage product C4c.
- 40. A kit according to claim 33 for detecting L-ficolin complex comprising:
  - (a) LTA immobilised on a support, and
  - (b) a reagent or reagents for detection of L-ficolin complex–LTA binding, and
  - (c) optionally, standard serum or purified L-ficolin/MASP complex or purified L-ficolin, suitable for generation of a standard curve, and
  - (d) optionally, instructions for use of the kit
- 41. A kit according to claim 40 wherein a reagent for detection of L-ficolin-LTA binding comprises a ligand capable of being labelled directly or indirectly with a detectable marker.
- 42. A kit according to claim 41 wherein the ligand is an antibody or a binding fragment of an antibody

WO 2005/051662

43. A kit according to claim 42 wherein the antibody or binding fragment of an antibody is specific for L-ficolin.

28

- 44. A kit according to claim 43 wherein the antibody is GN4 or GN5 or is a binding fragment thereof that specifically binds L-ficolin.
- A kit according to any one of claims 35 to 39 or claims 41 to 44 wherein 45. the ligand is labelled directly with a detectable marker.
- 46. A kit according to any of claims 35 to 39 or claims 41 to 45 wherein the ligand is labelled indirectly with a detectable marker.
- 47. A kit according to any of claims 35 to 39 or 41 to 46 wherein the detectable marker is a fluorescent, luminescent or radioactive marker.
- 48. A kit according to any of claims 35 to 39 or 41 to 47 wherein the detectable marker is selected from the group comprising alkaline phosphatase, radish peroxidase, biotin, europium horse (e.g. for time resolved immunofluorometric assays, TRIFMA), fluorescein isothiocyanate, a fluorescent protein or a radiolabel.
- A kit according to any of claims 35 to 39 or 41 to 48 wherein the 49. detectable marker is alkaline phosphatase and optionally a colorimetric substrate for detection of alkaline phosphatase is provided, preferably the colorimetric substrate is p-nitrophenyl phosphate (pNPP).
- 50. A kit according to any of claims 35 to 39 or 41 to 48 wherein the detectable marker is fluorescein isothiocyanate.
- 51. A kit according to any of claims 33 to 50 wherein the support is one or more wells on a multiwell plate.